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Previews

GABAergic synaptogenesis: a case for cooperation

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Abstract

Multiple cell adhesion molecules contribute to synapse formation by mediating transsynaptic interactions with presynaptic signalling molecules. In this issue of Neuron, Li et al. report cooperativity between Neuroligin2 and Slitrk3, exerting distinct effects on GABAergic synapse formation in immature and mature neurons.

Much attention has been given to the molecular mechanisms governing glutamatergic synapse formation and plasticity, based on the assumption that excitatory plasticity underlies learning, memory and higher cognitive functions. GABAergic synapses have long been considered to be stable elements controlling overall neuronal excitability by mediating shunting inhibition and hyperpolarization upon activation of $GABA_A$ receptors ($GABA_AR$). However, recent studies suggest that GABAergic transmission exerts more fundamental effects on neuronal networks by controlling spike timing and temporal fidelity, as well as rhythmicity of neuronal ensembles. In addition, GABAergic transmission can no longer be considered solely inhibitory, since Cl^- efflux which itself is dependent on the expression and activity of cation'chloride transporters, is depolarizing. These findings have profoundly influenced the consideration given to GABAergic transmission in adult and developing CNS. It is now widely accepted that GABAergic synapses are functionally heterogeneous, precisely

determine the function of neuronal networks, and control neuronal development and differentiation during ontogeny and in adult neurogenesis (Fritschy and Panzanelli, 2014). Evidence is increasing that perturbations of GABAergic synapse formation and function underlie major neurological, neurodevelopmental and psychiatric diseases, and that prevention and treatment of these diseases will depend, at least in part, on restoration of GABAergic function and/or inhibitory/excitatory balance. The study by Li et al. (2017) in this issue of Neuron adds a major novel element to our understanding of the mechanisms of GABAergic synapse formation by revealing, for the first time, a direct extracellular protein-protein interaction between two postsynaptic cell adhesion molecules, Neuroligin 2 (NL2) and SLIT and NTRK-like protein 3 (SLITRK3; ST3)—without interfering with the binding of each postsynaptic molecule to their canonical presynaptic partners, neurexins and Protein Tyrosine Phosphatase δ (PTBδ), respectively—in turn exerting differential, sometimes cooperative effects on GABAergic synapse formation depending on the developmental stage of the system.

In the past two decades, the key role of neuroligins and their presynaptic interaction partners neurexins for regulating GABAergic and glutamatergic synaptogenesis has been extensively studied. Likewise, the members of the SLITRK protein family, comprised of 5 members, also mediate GABAergic and glutamatergic synapse formation. Interestingly, loss of neuroligins or SLITRKs is not sufficient to fully abolish GABAergic synapse formation (Varoqueaux et al., 2006, Takahashi et al., 2012). Li et al. (2017) investigate the potential interaction between NL2 and ST3, which are both specifically enriched at GABAergic synapses, in regulating the formation of these synapses.

The authors use a series of experiments to uncover the relationship of NL2 and ST3 in GABAergic synapse formation. Central to the paper are the use of primary neuron cultures, as well as co-cultures between neurons and non-neuronal cells, amenable for screening and characterizing novel molecules involved in synapse formation and maintenance. Despite disruption of 3D network architecture and neuron-glia interactions, major features of neuronal maturation and circuit formation, including gene expression patterns, subcellular protein targeting, and mechanisms of synaptic transmission are preserved *in vitro*, and neuronal maturation is essentially complete within 2-3 weeks. Indeed, pure cultures of glutamatergic or GABAergic neurons forming autapses have shown that synaptogenesis relies on cell autonomous mechanisms ensuring proper differentiation of pre- and

postsynaptic machineries. However, a mismatch of $GABA_AR$ recruitment to glutamatergic synapses can be observed in pyramidal cells devoid of GABAergic input, indicating that proper functional specification requires the presence of interneurons (Fritschy and Brünig, 2003).

In addition to using primary neuronal and heterologous neuronal / non-neuronal co-cultures to test the cooperative role of NL2 and ST3 in the differentiation of presynaptic elements, the authors also silenced NL2 during early neuronal development in mice to find that loss of NL2 reduces GABAergic synapse function while specifically upregulating ST3 expression. Importantly, they combined immunohistochemical visualization of synaptic proteins with electrophysiological recordings to distinguish between synapse formation and function (which depends on the presence of postsynaptic $GABA_AR$) to show that NL2 and ST3 regulate synapse formation.

In brief, the study found that: 1) NL2 and ST3 regulation of GABAergic synapse formation depends on their intracellular C-terminal domain; in particular, the gephyrin- and the collybistin-binding domains of NL2, in immature neurons. 2) ST3 is required in combination with NL2 in mature neurons for inhibitory synapse development, in line with ST3's delayed expression during brain development. 3) NL2 enhances cell surface expression of ST3 via a nanomolar affinity interaction with the LRR9 motif of the LRRC2 domain of ST3, located in its extracellular, N-terminal domain, 4) the LRR9 motif is not necessary for ST3's synaptogenic function but the LRR9 motif allows for super-additive (cooperative) effects on GABAergic synapse formation, likely by enhanced cell surface expression of ST3 in the presence of NL25) mice specifically lacking the LRR9 motif in ST3, exhibit a deficit in GABAergic synapse formation, causing impaired oscillations of hippocampal neuronal networks and increased seizure susceptibility.

A particularly interesting aspect that emerges from the study is the temporal dependence of the function of ST3. ST3's expression in the brain and in cultured hippocampal neurons is delayed compared to NL2. Knockdown of NL2, but not of ST3, in neurons cultured for two days in vitro (DIV) largely eliminated mIPSCs and could be rescued with over-expression of either NL2 or ST3. When the same experiments were performed in DIV10 neurons, knockdown of either NL2 or ST3 strongly reduced mIPSCs in these more mature neurons. Knockdown of both genes eliminated nearly all mIPSCs and they could only be rescued fully by over-expression of both NL2 and ST3, whereas over-expression of NL2 alone was ineffective and over-expression of ST3 only partially rescued mIPSCs.

This temporal variation in dependence on NL2 and ST3 complements recently published observations that the collybistin (CB) splice isoforms CB1 and CB2 also have temporally-dependent functions, due in part to regulation of alternative splicing and distinct subcellular location. CB1 is expressed early during postnatal development, and influences GABAergic synapse formation *in vitro* along the proximal-distal axis of dendrites (de Groot et al., 2017). CB2 expression is not developmentally regulated, and it forms no proximal-distal gradient of GABAergic synaptogenesis. Li et al. (2017) report that in DIV8 neurons, the CB-binding domain of NL2 is required for GABAergic synaptogenesis; in contrast, in DIV18 neurons NL2 interaction with ST3 is essential for GABAergic synaptogenesis. A possible explanation for this observation could be that CB1 interaction with NL2 in developing neurons is sufficient to drive GABAergic synapse maturation, whereas in mature neurons, CB2 interacts with the NL2/ST3 complex to facilitate GABAergic synapse maturation (Fig. 1).

In vivo, temporal regulation of synaptogenesis might also underlie differential subcellular localization of GABAergic synapses (soma, proximal dendrites, distal dendrites), which are often defined by innervation by specific interneuron subpopulations in the hippocampus. For example, somatostatin-expressing "OLM" interneurons only target distal dendrites of hippocampal pyramidal cells, forming their synapses at a time when ST3 expression would be high (and CB1 expression low), whereas basket cells target the proximal compartment (soma and proximal dendrites).

In fact, Li et al. found that in mature neurons, the gephyrin- and CB-binding domains of NL2 are dispensable for GABAergic synapse formation, while the ST3 N-terminus is required. This suggests that ST3 harbors the ability to activate parallel yet currently unknown downstream mechanisms to recruit and assemble postsynaptic proteins, including $GABA_A$Rs in nascent synapses. Two reports recently characterized GARLH4 (also known as LHFPL4) as an essential recruiter and stabilizer of $GABA_A$Rs to inhibitory synaptic sites via its interaction with NL2 (Davenport et al., 2017; Yamasaki et al., 2017). GARLH4 function for $GABA_A$R synapse recruitment is restricted to principal cells, and, importantly, presynaptic GABAergic terminals (positive for vGAT) are intact in GARLH4$^{-/-}$ mice, suggesting that it does not mediate synapse assembly *per se*. Further, cell surface trafficking of $GABA_A$Rs is not disrupted in these mutants, but $GABA_A$Rs are no longer recruited to, or trapped, at postsynaptic sites. Therefore, GARLH4, likely acting with NL2, is selectively involved in postsynaptic protein clustering. Hence, one can envision two different scenarios for GABAergic synapse assembly in young versus mature pyramidal cells (Fig. 1): 1) In young

neurons still lacking ST3 expression, NL2 interaction with neurexin promotes GABAergic synaptogenesis. Synapse stabilization requires interaction of NL2 with GARLH4, followed by NL2-dependent CB and gephyrin postsynaptic recruitment, along with $GABA_A$Rs. 2) In mature neurons, ST3 is expressed and its cell surface expression and binding to the presynaptic receptor PTPδ is favored by interaction with the extra-cellular domain of NL2; possibly requiring PTPδ function, ST3 activates downstream pathways in addition to (or instead of) NL2, facilitating GARLH4, CB and gephyrin clustering, thereby resulting in enhanced $GABA_A$R trapping at synaptic sites. In the absence of NL2, this process is less effective, but is not abrogated.

In their study, Li et al. (2017) postulate that NL2-ST3 interaction represents "a general molecular mechanism underlying hippocampal inhibitory synapse development". However, neuron subtype-specific functions for synaptogenic molecules are not uncommon. Recently, as noted above, GARLH4 was shown to promote postsynaptic recruitment of $GABA_A$Rs specifically in pyramidal neurons (Davenport et al., 2017). IgSF9b has also been reported to be an interneuron-specific synaptogenic molecule (Woo et al., 2013) that acts indirectly with NL2 via a link involving S-SCAM. In this neuron subtype, initial recruitment of NL2 induces S-SCAM and IgSF9b clustering, gephyrin scaffold recruitment, and strengthening of synaptic adhesion. Finally, neuron type-specific mRNA splicing of neurexin isoforms also contributes to the diversity of interactions with CAMs (Nguyen et al., 2016). Hence, differential neurexin splicing has the potential to influence biochemical interaction with distinct postsynaptic partner proteins. For example, neurexin splicing might select for NL2/ST3 or NL2/IgSF9b interaction complexes, thereby differentially influencing GABAergic synaptogenesis and possibly postsynaptic recruitment of $GABA_A$R in different cell types. Such a mechanism would allow for the assembly of $GABA_A$R subtypes, distinguished by their subunit composition, with cell- and synapse-specific distribution.

The study by Li et al. (2017) in this issue of Neuron sheds light on several important novel features of GABAergic synapse formation: the hierarchical dependence on CAMs for synapse formation in development and the synergistic relationship between CAMs as exemplified by the described NL2-ST3 cis-interaction of their extracellular domains, which may apply for various types of synapses in hippocampal pyramidal cells and possibly all cortical principal cells. However, it is important to note that whether these features constitute a generalized mechanism remains unclear, since several conspicuous cell types (e.g.,

olfactory bulb mitral cells, Purkinje cells and stellate cells in the cerebellum) lack ST3 mRNA expression according to the Allen Brain Atlas. In addition, it should be considered that GABAergic synapse formation and elimination are on-going processes in adult brain, modulated by activity-dependent mechanisms and by signaling pathways that target post-translational modification of gephyrin and/or are controlled by metabolic activity to trigger synapse formation in the time scale of minutes (Tyagarajan and Fritschy, 2014). Whether these processes also involve NL2-ST3 interaction remains to be determined. Finally, the study by Li et al. (2017) also underscores the robustness of the overall molecular machinery of GABAergic synapse transmission. Deletion or silencing of single critical synaptogenic genes might reduce the formation or density of GABAergic synapses and affect the frequency and amplitude of synaptic events. However, there is considerable molecular redundancy, and, as seen best in NL2-null mice, or here in $ST3^{\Delta LRR9/\Delta LRR9}$ mice, these alterations do not preclude normal brain development or cause major behavioral deficits. Mutations identified in specific brain diseases rarely cause full functional inactivation (or dominant-negative) effects; yet, they can have serious pathophysiological consequences. Furthering the understanding of molecular mechanisms at work in immature and mature neurons and brain circuits—as seen in the work by Li et al. in this issue of *Neuron* examining developmental effects of NL2 and ST3—may give insights into effective therapeutic interventions in diseases associated with GABAergic dysfunction or excitation/inhibition imbalance with either early postnatal or adult onset.

References

Davenport, E., Pendolino, V., Kontou, G., McGee, T., Sheehan, D., López-Doménech, G., Farrant, M., and Kittler, J. (2017). An Essential Role for the Tetraspanin LHFPL4 in the Cell-Type-Specific Targeting and Clustering of Synaptic $GABA_A$ Receptors. Cell Rep 21, 70-83.

de Groot, C., Floriou-Servou, A., Tsai, Y., Früh, S., Kohler, M., Parkin, G., Schwerdel, C., Bosshard, G., Kaila, K., Fritschy, J., and Tyagarajan, S. (2017). RhoGEF9 splice isoforms influence neuronal maturation and synapse formation downstream of α2 $GABA_A$ receptors. PLoS Genet Oct 25;13(10).

Fritschy, J.M., and Brünig, I. (2003). Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. Pharmacol Ther 98, 299-323.

Li, J., Han, W., Pelkey, K.A., Duan, J., Mao, X., Wang, Y., Craig, M.T., Dong, L., Petralia, R.S., McBain, C.J., and Lu, W.Y. (2017). Molecular dissection of Neuroligin2 and Slitrk3 reveals an essential framework for GABAergic synapse development. Neuron in press.

Nguyen, T., Schreiner, D., Xiao, L., Traunmüller, L., Bornmann, C., and Scheiffele, P. (2016). An alternative splicing switch shapes neurexin repertoires in principal neurons versus interneurons in the mouse hippocampus. Elife 5, e22757.

Takahashi, H., Katayama, K., Sohya, K., Miyamoto, H., Prasad, T., Matsumoto, Y., Ota, M., Yasuda, H., Tsumoto, T., Aruga, J., and Craig, A.M. (2012). Selective control of inhibitory synapse development by Slitrk3-PTPδ trans-synaptic interaction. Nat Neurosci 15, 389-398.

Tyagarajan, S.K., and Fritschy, J.M. (2014). Gephyrin, a master regulator of neuronal function? Nature Rev Neurosci 15, 141-156.

Varoqueaux, F., Aramuni, G., Rawson, R.L., Mohrmann, R., Missler, M., Gottmann, K., Zhang, W., Suedhof, T.C., and Brose, N. (2006). Neuroligins determine synapse maturation and function. Neuron 51, 741-754.

Woo, J., Kwon, S., Nam, J., Choi, S., Takahashi, H., Krueger, D., Park, J., Lee, Y., Bae, J., Lee, D*., et al.* (2013). The adhesion protein IgSF9b is coupled to neuroligin 2 via S-SCAM to promote inhibitory synapse development. J Cell Biol 201, 929-944.

Yamasaki, T., Hoyos-Ramirez, E., Martenson, J., Morimoto-Tomita, M., and Tomita, S. (2017). GARLH Family Proteins Stabilize $GABA_A$ Receptors at Synapses. Neuron 93, 1138-1152.